

## Mass Spectrometric Detection of High-Fructose Corn Sirup in Honey by Use of $^{13}\text{C}/^{12}\text{C}$ Ratio: Collaborative Study

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Mass spectrometry is used to determine the  $^{13}\text{C}/^{12}\text{C}$  ratios in honey products. The  $^{13}\text{C}/^{12}\text{C}$  ratios in domestic and imported honeys are sufficiently consistent and different from those of high-fructose corn sirups (HFCSs) so that the presence of added HFCS in honey can be demonstrated. Average values of  $\delta^{13}\text{C}$  of  $-25.4\text{‰}$  (parts per thousand) for 119 honey samples and  $-9.7\text{‰}$  for 4 HFCSs were found.  $\delta^{13}\text{C}$  values of mixtures are the weighted sum of the  $\delta^{13}\text{C}$  values of the components. In a collaborative study, 4 honey-HFCS mixtures and 1 pure honey sample were correctly identified by 6 laboratories. The method has been adopted as official first action.

The relatively high price, limited supply, and complexity of honey combine to encourage falsification in part or *in toto*. Until recently the adulterants were conventional corn sirup and commercial invert sirup, both of which may be detected by existing methodology (1).

A major development in bound enzyme technology has enabled production of a new industrial sweetener, high-fructose corn sirup (HFCS). The solids of this low-cost, highly refined sirup consist of about 50% glucose and 42% fructose, with small amounts of higher saccharides. Refining by activated carbon and ion exchange leaves little other material. There is concern that HFCS will be used to adulterate honey.

Because of this concern, we investigated methods to detect HFCS in honey. The crucial importance to our economy of pollination of food, fiber, and seed crops requires that confidence in the honey market, the beekeeper's principal income source, be restored and maintained.

The large variability among the known constituents of honey makes the use of ordinary compositional parameters most difficult. An ap-

parently attractive approach has been the ratios of potassium and sodium in honey and HFCS. Honey is high in potassium, and HFCS is high in sodium because of the ion exchange purification. A recent proposal (2) involving this principle has been shown not to be sufficiently selective to be useful (3).

Several other possibilities are under study and will be reported in other papers. The principal difficulty has been the varying degree of purity among the HFCSs from the 7 current United States producers. Some HFCSs may be detected easily while others do not respond to proposed tests.

Nissenbaum *et al.* (4) suggested that the  $^{13}\text{C}/^{12}\text{C}$  ratio may be useful to detect falsification of various types of plant products, using materials derived from corn. It has been known for some years that carbon isotope fractionation is associated with photosynthesis. Smith and Epstein (5) and Bender (6) reported that higher plants fall into 2 categories with respect to the carbon isotope ratio: Plants using the Calvin photosynthetic cycle have  $\delta^{13}\text{C}$  values of  $-22$  to  $-33\text{‰}$  (parts per thousand) and those using the Hatch-Slack  $\text{C}_4$  cycle have  $\delta^{13}\text{C}$  values in the  $-10$  to  $-20\text{‰}$  range. Corn is in the latter group; most dicotyledonous plants are in the former.

We found that the presence of corn products in honey can be unequivocally shown by measuring the stable carbon isotope ratio by mass spectrometry. The procedure was collaboratively tested as described below.

### Experimental

#### Materials and Methods

United States honey samples were from domestic honey producers; a certification of authenticity accompanied each. Different imported honey samples were selected from those submitted by 3 United States honey importers, representing trade samples and subsamples from actual shipments.

J. W. White, Jr received the 1977 Associate Referee of the Year Award.

Table 1.  $\delta^{13}\text{C}$  of honeys and HFCSs<sup>a</sup>

Source	No. of samples	Mean, ‰	Range		Std dev., ‰	Coeff. of var., %
			High	Low		
United States honey	84 <sup>b</sup>	-25.2	-22.5	-27.4	0.94	3.73
Imported honey	35	-25.8	-23.9	-27.4	0.97	3.76
All honey	119	-25.4	-22.5	-27.4	0.98	3.86
HFCS	4	-9.7	-9.5	-9.8	0.14	1.4

<sup>a</sup> See refs 7 and 8 for individual values.<sup>b</sup> Includes 4 honeydew honey samples of positive polarization.

### Determination of $\delta^{13}\text{C}$

Carbon isotope ratios were determined by Geochron Laboratories Division, Krueger Enterprises, Inc., Cambridge, MA 02139, on an AEI MS-20 double-collecting, 180° sector mass spectrometer with a dual capillary inlet.

The usual corrections were applied to the measured differences, including any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, tailing of major onto minor peak signal, and contribution of  $^{17}\text{O}$  to mass 45 signal. Samples were combusted in purified oxygen at about 850°C and the gases were recirculated over CuO at 850°C for 10 min; water and CO<sub>2</sub> were frozen, excess oxygen was removed, and CO<sub>2</sub> was transferred to a sample flask for analysis. Results are expressed in  $\delta^{13}\text{C}$  units, compared to a reference standard of Pee Dee belemnite (PDB). Geochron Laboratories stated reproducibility for the system was 0.3‰; however, results for the 5 duplicate honey samples agreed within 0.1‰, and results for the analysis of the crude oil reference samples also agreed within 0.1‰.

The following experiments were conducted to establish the applicability of this procedure:

(1) The range and variability for  $\delta^{13}\text{C}$  values in United States and imported honey and HFCS were established, since no information was available in the literature.

(2) The relationship between  $\delta^{13}\text{C}$  and the composition of mixtures of honey and HFCS was determined.

(3) Samples of known composition were subjected to collaborative testing.

### $\delta^{13}\text{C}$ Values in Honey and HFCS

From nearly 500 samples from United States producers, 80 samples of honey and 4 samples of honeydew honey (identified by positive polarization) were selected to represent the distribution of United States commercial honey as well as

whole season mixtures from wide geographic areas. Details on sample selection and analytical results for individual samples are reported elsewhere (7, 8). Four samples of HFCS from 4 United States manufacturers were also analyzed for  $\delta^{13}\text{C}$ . The values are summarized in Table 1; their distribution is shown in Fig. 1.

To apply the isotope ratio test to honeys of foreign origin, the ranges of values for the types of honey commonly imported into the United States were determined. Environmental effects on the ratio have been reported (5, 6).

Two importers and an importer-packer supplied 46 honey samples representing all significant foreign sources of honey. These included trade samples intended to represent lots offered for sale and samples from actual shipments. After duplica-

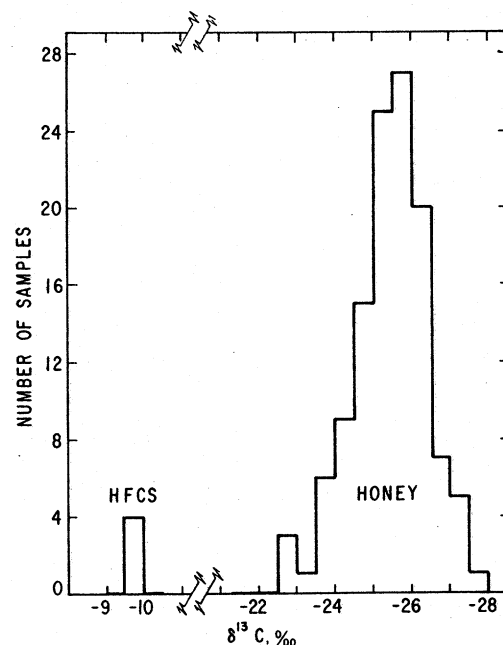


FIG. 1.—Distribution of  $\delta^{13}\text{C}$  values among 4 HFCSs and 119 samples of honey, including 4 honeydew honeys.

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

**Table 2. Probability of the  $\delta^{13}\text{C}$  value of an authentic honey sample being more negative than a stated limit**

Probability, %			Limit of $\delta^{13}\text{C}$ , ‰
5 of	6	84.1	-24.4
43 of	44	97.72	-23.4
769 of	770	99.87	-22.5
24,999 of	25,000	99.996	-21.5

**Table 3.  $\delta^{13}\text{C}$  (‰ vs. PDB) values for collaborative samples**

Coll.	Sample				
	A	B	C	D	E
1a <sup>a</sup>	-17.6	-14.0	-19.3	-15.2	-25.1
1b <sup>a</sup>	-17.4	-13.5	-19.5	-14.8	-24.8
2	-17.4	-13.8	-19.5	-15.1	-25.1
3	-17.7	-13.9	-19.8	-14.7	-24.4
4	-17.7	-13.6	-19.3	-15.0	-24.9
5	-18.5	-14.9	-21.4	-16.0	-25.9
6 <sup>b</sup>	-17.1	-13.4	-19.3	-14.6	-24.9
Mean	-17.63	-13.87	-19.72	-15.06	-25.01

<sup>a</sup> The second set of values were obtained 5 months after the first set, using another instrument.

<sup>b</sup> These results were obtained 5 months after the rest of the results.

Imples and samples from latitudes already represented in the United States honey study were removed,  $\delta^{13}\text{C}$  values were determined for 35 samples from 15 countries; see Table 1. The individual values are reported elsewhere (8).

#### Relationship of $\delta^{13}\text{C}$ and Composition of Mixtures

The following 3 sets of mixtures were prepared, with honeys and HFCSs of known  $\delta^{13}\text{C}$ : (A) HFCS  $\delta^{13}\text{C}$  -9.5‰, honey  $\delta^{13}\text{C}$  -26.0‰, 5 mixtures; (B) HFCS  $\delta^{13}\text{C}$  -9.8‰, honey  $\delta^{13}\text{C}$  -23.0‰, 5 mixtures; (C) repetition of B with 4 mixtures, made in reverse order from B. The mixtures were analyzed without knowledge of composition by Geochron Laboratories. For calculation, the data from mixtures B and C were combined. The regression equations for the 2 sets of mixtures were:

$$\delta^{13}\text{C mixture (A)} = -10.09 - 0.1601H$$

$$\delta^{13}\text{C mixture (B or C)} = -10.06 - 0.1316H$$

where  $H$  is the per cent honey in the mixture.

Correlation coefficients of -0.997 and -0.998 were obtained for mixtures A and B + C, respectively;  $F$  values in the analysis of variance were 993 and 5450, respectively, which are significant at the >99.9% probability level. Thus the  $\delta^{13}\text{C}$  value of a mixture of HFCS and honey is the

sum of the fractional contribution of each component, as expected.

There is a degree of uncertainty in the use of this test to prove the presence of HFCS in honey because of the wide range of values found for honey. The upper (least negative) limit for  $\delta^{13}\text{C}$  for authentic honey may be set with any desired degree of certainty as the mean plus a multiple of  $S$ , as shown in Table 2.

Measurement of stable isotope ratios with the precision and accuracy needed for this application requires specialized equipment not ordinarily found in control laboratories. To use the isotope ratio values for enforcement purposes, the precision and accuracy of the determination must be acceptable, including sample combustion and instrumental factors. Although laboratories capable of conducting this analysis use and exchange various reference materials and report results compared to PDB as a reference standard, PDB is no longer available.

#### METHOD

See secs 31.D01-31.D04 plus Fig. 31.D1, March issue.

#### Collaborative Study

Five samples were sent to 7 laboratories for the collaborative study. Sample E was pure honey. Samples A-D were mixtures containing 25-65% honey: Four different unanalyzed honeys were separately mixed with the same HFCS to produce a similar color for each of the different dilutions with colorless HFCS. The honey contents of Samples A-D were 50.83, 25.91, 65.50, and 34.13%, respectively.

#### Results and Recommendation

Useful results were obtained from 6 laboratories; the seventh was in the earlier stages of installation and calibration and all of the results reported differed greatly from the other collaborative results; they are not included in this article. Collaborator 1 also analyzed the samples 5 months later with a newly acquired instrument; both sets of results are given in Table 3. Collaborator 6 analyzed the samples 5 months later than the other collaborators.

The 6 sets of individual  $\delta^{13}\text{C}$  values are given in Table 3. All of the samples were correctly identified, using any of the limits on Table 2. Although the results from Collaborator 5 were

The recommendation of the Associate Referee was approved by the General Referee and by Subcommittee D and was adopted by the Association. See (1978) *JAOAC* 61, 394.

different from the other collaborative results and the differences approached the 4S limit for Sample C, he correctly identified all samples. His results were also repeatable within  $\pm 0.3\%$ , compared to the  $\pm 0.1\%$  reproducibility for the other collaborators.

The results in Table 3 were examined statistically using the procedures recommended by Youden (10). For this analysis, Sample A was paired with C and B with D. Results are given in Table 4. Since all collaborators correctly identified all samples, the table shows this treatment for all results; the significant  $F$  value indicates systematic error to be present. Application of Youden's ranking test excludes Collaborator 5. Recalculation without those results improves the values sufficiently to eliminate significant systematic error.

To relate the data in Table 3 to actual composition of samples, a single regression equation was calculated using data from the 14 mixtures used for linearity testing. Using this equation, the compositions corresponding to the mean values in Table 3 were calculated and are shown in Table 5, together with the actual composition (by weight) of the collaborative samples. Excellent agreement was obtained, considering that none of the 4 honeys used was included in the linearity test.

**Table 4. Statistical evaluation of data in Table 3**

Statistic	Pairs	
	B-D	A-C
All Collaborators		
$S_d$	0.673	0.825
$S_r$	0.138	0.297
$S_b$	0.466	0.544
$F^a$ (6 DF)	23.71**	7.70**
Excluding Collaborator 5		
$S_d$	0.300	0.265
$S_r$	0.148	0.177
$S_b$	0.184	0.140
$F^a$ (5 DF)	4.09	2.25

<sup>a</sup> For presence of systematic errors. DF = degrees of freedom.

**Table 5. Composition of test samples (% honey)**

Composition	A	B	C	D	E
Known	50.8	25.9	65.5	34.1	100
Found <sup>a</sup>	50.5	25.4	64.4	33.3	99.7

<sup>a</sup> Using average of all values in Table 3 and equation derived from mixtures used for linearity test.

The various instruments and conditions used in the collaborative study are given in Table 6. In addition, all collaborators applied the usual corrections: for zero enrichment in the inlet sys-

**Table 6. Instruments and conditions used in the collaborative study**

Coll. <sup>a</sup>	Mass spectrometer	Combustion <sup>b</sup>			Working std <sup>c</sup>
		Temp., °C	Recirculation time, min		
1a	AEI-MS double collecting 180° sector type, with dual capillary inlet	850	10		charcoal ( $-23.3\text{‰}$ PDB) and marble ( $0.0\text{‰}$ PDB) calibrated against NBS-20 Solenhofen limestone ( $\delta_{13} = -1.06\text{‰}$ vs. PDB) and NBS-22 (crude oil, $-29.5\text{‰}$ )
1b	Micromass 602D	850	10		same as 1a
2	Micromass 602C, adapted to analysis of stable isotopes	1200	—		unspecified std calibrated against PDB
3	Nier 60° sector type, modified according to ref. 9	800-900	30		unspecified
4	Nuclide 6 in. ratio type, with digital integrating radiometer	900 (CuO at 700)	—		NBS-21 graphite ( $-27.8\text{‰}$ ) and NBS-22 oil ( $-29.5\text{‰}$ ), NBS-20 Solenhofen limestone as instrument std
5	Nuclide 6-60 RMS	600-800	30		instrument std calibrated against NBS-20 Solenhofen limestone

<sup>a</sup> Collaborator 6 did not supply this information.

<sup>b</sup> Sample was combusted in ca 60 cm O<sub>2</sub> pressure in manifold in tubular furnace and gases were recirculated over hot CuO. Water was condensed at  $-79^\circ\text{C}$  and CO<sub>2</sub> was removed at liquid air temperature, and excess oxygen was removed. Collaborator 2 did not use CuO; he relied on the high temperature for complete combustion.

<sup>c</sup> NBS = National Bureau of Standards reference material.

tem, mixing between sampling and standard values, tailing of major onto minor peak signal, and contribution of  $^{17}\text{O}$  to mass 45 signal.

On the basis of the results reported here, the Associate Referee recommends that the mass spectrometric determination of  $^{13}\text{C}/^{12}\text{C}$  ratio be adopted as official first action for detecting the presence of HFCS in honey.

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